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Nutritional ecology and the evolution of aging

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ABSTRACT

Considerable progress has been made in understanding both evolutionary and mechanistic aspects of biological aging, although the two areas remain poorly integrated. We suggest that a greater emphasis on ecology can help to remedy this, by focusing on the interface between biological mechanisms and the environments in which they evolved by natural selection. Among the most salient aspects of the environment relevant to aging is nutrition, and yet in the bulk of aging research nutrition is coarsely represented as dietary restriction or caloric restriction, without consideration for how specific components of diet, beyond "energy" (the undifferentiated mix of macronutrients), are driving the observed effects. More recently, it has become clear that specific nutrients (notably amino acids) and interactions among nutrients (i.e., nutritional balance) play important roles in the biology of aging. We show how a method developed in nutritional ecology, called the Geometric Framework for nutrition, can help to understand the nutritional interactions of animals with their environments, by explicitly distinguishing the roles of calories, individual nutrients and nutrient balance. Central to these models are the active regulatory responses that animals use to mediate between variation in the nutritional environment and fitness-related consequences such as lifespan and reproduction. These homeostatic responses provide a guide for researchers that can help to link the biological mechanisms with evolutionary processes in the context of a multi-dimensional nutritional environment.

1. Introduction

The almost universal phenomenon of aging is considered one of the enduring unsolved problems in biology (Dev, 2015). Broadly, the study of biological aging has clustered within two general areas (Hughes and Reynolds, 2005; Monaghan et al., 2008; Flatt and Schmidt, 2009). One sub-field applies evolutionary theory and techniques to understand how a process so detrimental to health, reproduction and survival can be reconciled with evolution by natural selection. The other is mechanistic, aiming to understand the underlying molecular, cellular and physiological processes, often in laboratory studies using experimental model systems. Although there have been significant advances over recent years both in evolutionary and mechanistic research into aging, important challenges remain. Arguably, however, none of these challenges considered separately would qualify for the list of major unsolved problems in biology (Hayflick, 2007a, b). Rather, as in other areas of life-history research, the big questions lie at the intersection of different sub-fields (Flatt and Heyland, 2011): how to reconcile the evolutionary and mechanistic theories of aging, and how to apply these theories and their reconciliation to ensure that gains in human healthspan are commensurate with gains in lifespan (Cesari et al., 2013).

Increasing attention is being focused on the question of how evolutionary and mechanistic strands of aging research can be integrated into a single framework to produce an over-arching theory of biological aging (Partridge and Gems, 2006; Flatt and Schmidt, 2009). An important, but under-represented area for addressing this challenge is the science of ecology. Ecology focuses both on the interactions that take place between organisms within ecological assemblages (broadly referred to as "community ecology", Stroud et al., 2015), and the details of how specific traits of organisms interact with biotic and abiotic aspects of the environment (sometimes referred to as "functional ecology", Calow, 1987; Raubenheimer and Boggs, 2009). Between functional and community ecology is "population ecology" (Krebs, 2015), which deals with questions regarding the distributions of populations of species in space and time. The functional-population-community ecology triumvirate is intimately associated via evolution (McLachlan and Ladle, 2011), because biological traits, such as the proximate factors influencing lifespan and the schedule of reproduction, evolve through differential success within populations in the context of community interactions (e.g., foraging and predation). Ecology therefore provides a body of theory that is essential for linking organism traits with evolution, as is recognised in the integrative field of evolutionary ecology (Fox et al., 2001; Cheplick, 2015).

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In many, if not most cases, ecology is already implicit in both mechanistic and evolutionary studies of aging. For example, the majority of experimental manipulations that generate variance in physiological responses emulate variance in the environment (e.g., dietary restriction); likewise, many of the processes that evolutionary theories of aging address are ecological — prominent among these are the risk of extrinsic mortality (Shokhirev and Johnson, 2014) and resource availability (Kirkwood, 1977). Ecology can thus provide a substantial link to bridge mechanistic and evolutionary theories if greater emphasis is placed on exploring the multi-faceted and dynamic interface between evolved mechanisms and the environment in which they operate.

The strong evidence that senescence is a real phenomenon in wild populations (e.g. Nussey et al., 2013) opens the way for a deeper integration of ecology with aging research. This has already begun, to the extent that in recent years there have appeared journal special issues dedicated to the subject (Monaghan et al., 2008; Fletcher and Selman, 2015). However, a large proportion of research into aging concerns nutrition, but the ecological aspects of the relationship between nutrition and aging remain scantly developed. In the most common model, dietary restriction, animals that are restricted in availability of macronutrients while provided sufficient micronutrients usually have extended lifespans relative to unrestricted controls (McCay et al., 1935). And yet the nutritional causes relating dietary restriction to senescence and lifespan are poorly understood. A widespread assumption is that energy is responsible, as suggested by the commonly used synonym for dietary restriction "caloric restriction" (Speakman and Mitchell, 2011). Some research, however, has implicated not calories per se, but the protein component, with particular roles for specific amino acids (Speakman and Mitchell, 2011; Fontana and Partridge, 2015). A more-detailed account of how diet impacts on aging is needed to understand the evolution of senescence, the underlying mechanisms, and the ecological contexts in which they evolved.

Over the past two decades the field of nutritional ecology has demonstrated across diverse taxa and contexts (e.g., lab experiments, free-living wildlife, animal production systems, companion animals) that animal-food interactions are complex, involving homeostatic regulatory mechanisms, such as appetite systems, that intricately mediate the relationships between many nutrients and their physiological impacts (reviewed in Simpson and Raubenheimer, 2012). A state-space modelling approach that has been developed to study these interactions, called the Geometric Framework for nutrition, has shown that their inclusion in empirical and theoretical studies can substantially increase predictive and explanatory power compared with studies based on a single currency, such as energy or protein (e.g., Raubenheimer, 2011; Raubenheimer and Simpson, 1993; Raubenheimer et al., 2009; Simpson and Raubenheimer, 2005, 2012; Simpson et al., 2015). The Geometric Framework has been applied in theoretical (Simpson and Raubenheimer, 2007; Simpson and Raubenheimer, 2009; Piper et al., 2011) and empirical research into aging, the latter including several insect species and one mammalian model system, C57BL6 laboratory mice (reviewed in Le Couteur et al., 2016).

Our aim in this paper is to introduce the logic of the Geometric Framework, and show in the context of experiments exploring links between nutrition, reproduction and aging how it can be used to develop a detailed understanding of the interface between the animal and its nutritional environment that could help to unify mechanistic and evolutionary theories of aging. Against this background, we discuss the ecological relevance of the caloric restriction protocol in aging research, and conclude that a broader paradigm is needed which considers the causal links between diet and lifespan to be an open-ended question. This will provide a stronger basis for integrating mechanistic and evolutionary theories of aging, and leave the way open for non-model organisms to contribute maximally to understanding biological aging.

2. The geometry of diet

We begin by showing how some core concepts in nutritional ecology are represented within the Geometric Framework. The device within this framework in which the interaction of the animal with its nutritional environment is modelled is a Cartesian space called a *nutrient space* (Fig. 1A). The two or more axes defining this space each represent a functionally important food component, for example the macronutrients protein, carbohydrate and fat. Within the nutrient space, key aspects of the environment (e.g., food compositions), the animal (e.g., current nutritional state, optimal nutritional state), and its interaction with the environment (feeding and other homeostatic responses) can be represented in common, multidimensional nutrient metrics.

Such a nutrient space representing the animal's nutritional interaction with the environment can be empirically parameterised either through captive animal experiments or observational studies in the wild, and related to various outcomes of interest, including mechanistic responses (e.g., activation of key nutrient signalling pathways and physiological markers of health and aging) and functional outcomes, such as reproduction and longevity. In this way nutritional geometry provides a template in which the animal's evolved responses to a multi-dimensional nutritional environment can reveal the links between functional outcomes and the underlying mechanisms, as we detail further in the rest of this section.

2.1. Homeostatic targets

A central tenet of nutritional ecology is that the interactions of animals with their environments are not passive, but actively guided by homeostatic systems that have evolved to produce adaptive outcomes. To model this, the nutritional states on which the animal will converge if unconstrained are represented within the nutrient space as target points or small regions. The *intake target* describes the cybernetic goal of the mechanisms regulating ingestion (Fig. 1A); physiological targets, for example the *growth target*, can similarly be described (Raubenheimer et al., 2009), but we will not consider these further in this paper.

An animal reaches its intake target through the selection of foods, and regulating how much of each is eaten. Foods are represented within the nutrient space as the ratio of the nutrients that each contains. Geometrically, this is given as the slope of a radial that projects from the origin into the nutrient space, called a *food rail*.

As the animal eats, it ingests the nutrients in the same proportion as they occur in the food, and consequently its nutritional state can be modelled as changing along the rail representing the food that it is eating - the more it eats, the further along the rail it "moves". If the rail representing a particular food intersects the intake target (i.e., contains the same ratio of nutrients that is prioritised by the animal's regulatory systems), then this food is nutritionally balanced with respect to the nutrients in the model, and by eating the right quantity of this food the animal can "navigate" directly to its intake target (e.g., Food 1 in Fig. 1A). By contrast, if the food is imbalanced then it does not on its own allow the animal to reach its target (e.g., Food 2). However, the animal can nonetheless use this food to navigate to the target, if it combines it in the diet with another imbalanced food, provided the two foods fall on opposite sides of the intake target (e.g., Food 2 combined with Food 3). Such combinations of nutritionally imbalanced foods that can be combined into a balanced diet are called *complementary* food pairings.

Importantly, regulatory targets like the intake target are not merely hypothetical constructs, but can readily be measured in laboratory studies or even in free-ranging wild animals (Felton et al., 2009a; Johnson et al., 2013; Raubenheimer et al., 2015). Experimentally, the protocol is to provide the animals with complementary food pairings and measure the point of intake on which the animal converges over a stipulated period. To ensure that this point does, in fact, represent a homeostatically regulated outcome, it needs to be statistically distinguished from a null hypothesis (Fig. 1B). This could be a mathematical expectation, for example observed intakes could be compared to the anticipated outcome if feeding were indiscriminate or random.

Alternatively, the test could be between two or more treatment groups each confined to a different complementary food pairing. The food pairings might be distinguished either by the ratios of the nutrients they contain, the concentrations of the nutrients, or both. Either way, in this design if the animals in different treatments spread their feeding similarly between the two foods in the respective food pairings the intake points for the treatment groups would fall in different regions in the nutrient space; in contrast, if they compensated for the differences between the respective pairings to converge on a common point in nutrient space this would suggest homeostatic regulation (Fig. 1B). The same logic applies for observational studies in the wild, except such studies rely on natural variation in the composition of available foods (Raubenheimer et al., 2015).

Intake targets are fundamental in geometric models, because they provide a direct measure of the evolved nutritional priorities of the animal. This is important in its own right, and as we demonstrate below also provides a powerful point of reference for understanding the regulatory responses of animals to dietary constraint, for example due to poor nutritional quality or restricted quantity of available foods, and the fitness-associated consequences for the animal of such constraint.

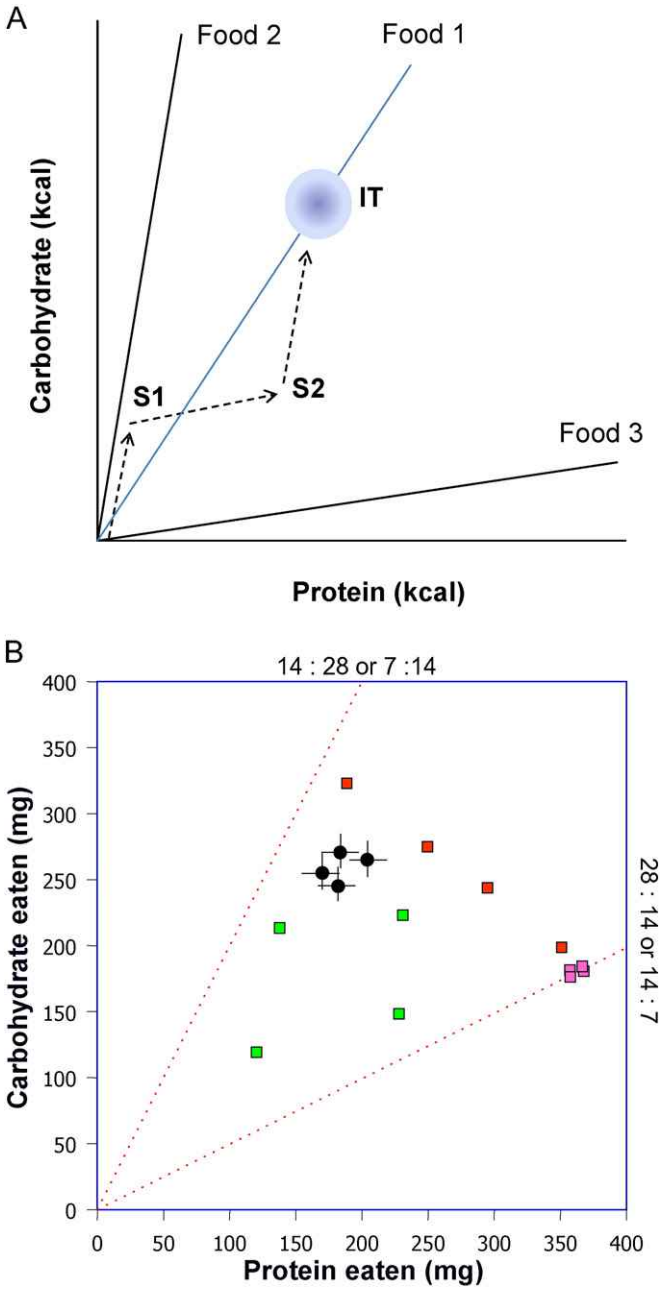


Fig. 1. A. Schematic illustrating nutrient regulation in relation to dietary balance in the Geometric Framework for nutrition, using a model involving a two-dimensional (protein and carbohydrate) nutrient space. The intake target (IT) represents the balance and amounts of macronutrients targeted by the regulatory mechanisms. Foods are represented by radials, called nutritional rails, projecting into the nutrient space at angles determined by the ratio of the nutrients they contain. As the animal eats, its nutritional state changes along a trajectory with the same slope as the nutritional rail for the food it is eating, with the distance moved along this trajectory being determined by the amount eaten. For example, by eating Food 2, the animal's state changes from the origin to state S1. The animal can achieve its target state by selecting Food 1, which is nutritionally balanced with respect to its target, or else by mixing its intake from nutritionally complementary foods 2 and 3. Thus, when in state S1 the animal is off-course in relation to its intake target, but by switching to Food 3 it can change to S2, and a further switch back to Food 2 would take it to the intake target. B. Geometric model showing ingestive regulation of protein and carbohydrate by 5th stadium locusts (*Locusta migratoria*) compared with intakes expected under three alternative hypotheses (data from Chambers et al., 1995. See also Raubenheimer et al., 2009). Animals were fed over 6 days on one of four food pairings composed of (% protein: % carbohydrate): 7:14 & 28:14; 7:14 & 14:7; 14:28 & 28:14; 14:28 & 14:7. Despite being given different food pairings, each group of locusts arrived at a similar mean selected intake (black dots with error bars) suggesting homeostatic regulation of macronutrient intake. Colored square symbols indicate predicted intake patterns under the following alternative hypotheses: regulation to a constant energy intake (red squares); regulation to maximize protein intake (pink squares); and equal intakes of the two options in each food pairing, indicating absence of macronutrient or energy regulation (green squares).

2.2. Dietary constraint

In an ecologically favourable situation an animal is able to select balanced foods and/or complementary pairings to satisfy its target nutrient intake as discussed above. In reality, however, ecological constraints on the quantity or quality of foods available might prevent the animal from achieving the homeostatic target. Constraints are termed *quantitative* if they involve restricted access to food. Under *qualitative* constraints, by contrast, foods are available, but these are imbalanced in ways that restrict animals from reaching their intake target.

Qualitative constraint is a more complex, and from a regulatory perspective more interesting situation than quantitative constraints. An animal restricted to non-complementary imbalanced foods, by definition, is forced into a situation where it cannot achieve the target intake for all nutrients, but is confronted with a trade-off between over-ingesting some and/or under ingesting others (Fig. 2A). Since both nutrient deficits and ingested excesses can have fitness costs (Simpson et al., 2004; Raubenheimer et al., 2005), and these costs will differ for animals in different circumstances, the regulatory responses underlying this trade-off are themselves subject to natural selection (Simpson and Raubenheimer, 2012). These responses, termed *rules of compromise* (Fig. 2B), are therefore secondary regulatory targets which can provide for researchers information about the relative priorities assigned by the animal to avoiding surpluses and deficits of specific nutrients in situations where ecological constraint prevents them from achieving the primary target.

Like intake targets, rules of compromise have been measured in a variety of animals and contexts, both in laboratory studies (reviewed in Simpson and Raubenheimer, 2012) and in the wild (Felton et al., 2009b; Rothman et al., 2011; Irwin et al., 2015). Together these homeostatic responses can yield useful insight into the evolved functional priorities of animals (Simpson et al., 2004), and also provide a systems-level description of the animal that can be used as a framework for understanding the underlying mechanisms. To establish these relationships we need to incorporate into the model variables that are measured in units other than nutrients, for example levels of signalling molecules, reproductive output and longevity, to relate to the underlying nutrition. Below we address the question of how such response variables are integrated into geometric models, using examples involving real data. First, however, we consider in more detail the concepts of

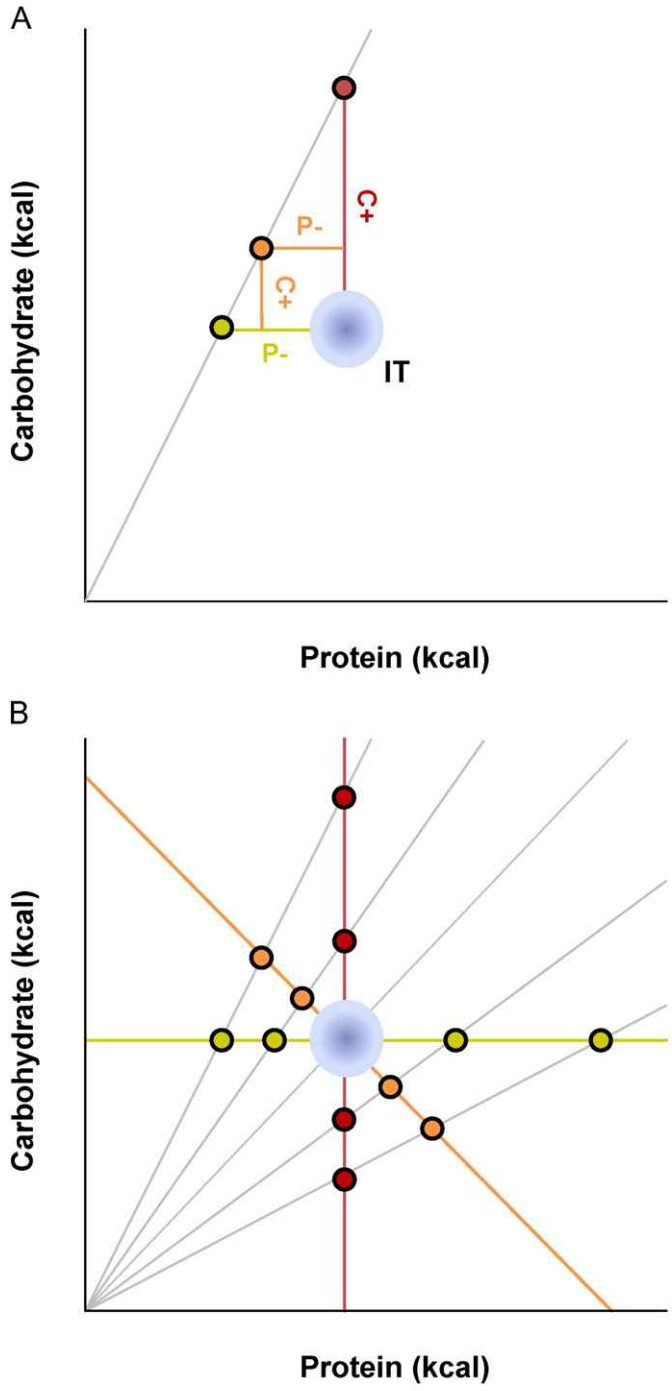


Fig. 2. Schematic illustrating nutrient regulation in relation to dietary imbalance in nutritional geometry, using a two-dimensional model (protein and carbohydrate). **A.** When confined to a single nutritionally imbalanced food (i.e., a food rail that doesn't intersect the intake target), the animal needs to resolve a trade-off between over-ingesting one nutrient and under-ingesting the other. By feeding to the yellow-green point it would meet its target for carbohydrate at the cost of a shortage of protein of magnitude P−, at the red-brown point it would meet its target for protein but over-ingest carbohydrate (C+) and at the orange point it would suffer both a shortage of protein and an excess of carbohydrate, but to a lesser extent than the yellow-green and red-brown points, respectively. B. Testing different experimental groups each on one of a range of foods varying in nutrient balance provides a description of how the animal resolves the trade-off between over- and under-ingesting nutrients when confined to imbalanced foods, termed a rule of compromise. Three possibilities are illustrated: the red-brown symbols represent absolute prioritisation of protein (i.e., feeding to the target coordinate for protein regardless of whether this involves over- or under-eating carbohydrate), the yellow-green symbols represent absolute carbohydrate prioritisation, and the orange symbols represent equal weighting of the two nutrients, as would be the case for energy prioritisation in which the animals feed to fixed energy intake (i.e., kcal carbohydrate + kcal protein intake = constant). Many other configurations are possible.

quantitative and qualitative constraint, and how these relate to the design of experiments investigating relationships between diet and lifespan.

3. Experimental considerations

3.1. Restricting what?

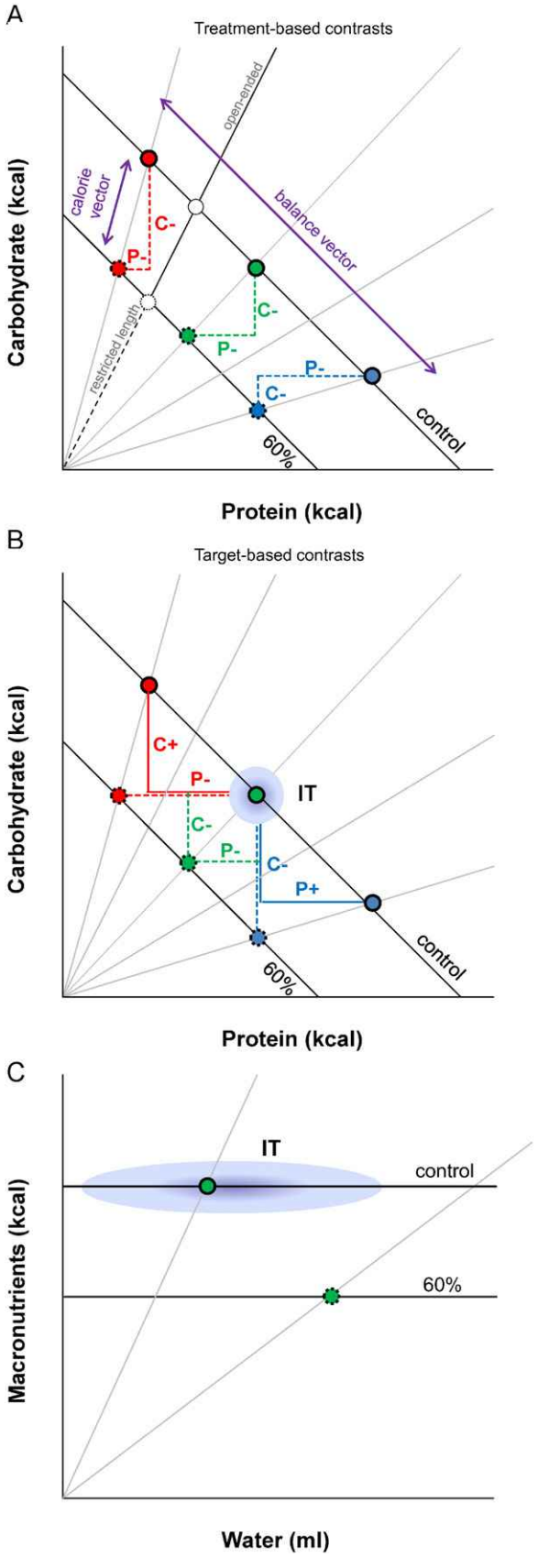
The nutrient space, food rails, intake targets, and rules of compromise provide a means to conceptualise in multiple nutritional dimensions the relationships between foods, diets and animal regulatory responses. They also provide a framework to define and experimentally emulate very specific nutritional scenarios, enabling the relationships between diet, mechanism and functional responses, such as lifespan and reproduction, to be explored.

The most commonly used manipulation in dietary restriction experiments is *quantitative restriction*, in which animals are provided with reduced rations of an experimental food. In the now widespread model termed "caloric restriction" (Speakman and Mitchell, 2011), micronutrient concentrations in the experimental foods are increased to help ensure that observed effects can be ascribed specifically to caloric intake (i.e., the undifferentiated mix of macronutrients), unconfounded by micronutrient deficiencies. Geometrically, this corresponds with manipulating the length of the rail representing the experimental food, thus limiting the distance the restricted animals can move radially from the origin into the nutrient space relative to the ad libitum group. This is illustrated by the short dashed food rail associated with the white treatment comparison in Fig. 3A, labelled "restricted length".

In contrast, *qualitative restriction by balance* is achieved through providing all treatment groups with unrestricted (i.e., ad libitum) access to one of a range of foods that vary systematically in the ratios of macronutrients. Geometrically, this corresponds with manipulating the angle of the nutritional rail (i.e., creating variation along the "balance vector" in Fig. 3A). Qualitative restriction can also be used to manipulate the distance moved by animals along nutritional rails (total calories eaten, shown as variation over the "calorie vector"). This is done by diluting the macronutrient content of the experimental foods using a filler, for example cellulose (Maklakov et al., 2008; Solon-Biet et al., 2014, 2015a) or water (Lee et al., 2008; Fanson et al., 2009), which has the effect of restricting the amounts of the nutrients eaten while providing the animals with ad libitum access to the foods. The extent to which the animals subject to this treatment are restricted in their intake of nutrients depends on the extent to which they compensate for dietary dilution through increasing food intake. This phenomenon, which we will call *qualitative restriction by dilution*, is illustrated by the dash-circled red, green and blue treatment groups in Fig. 3A. All three of these groups have access to the same amount of experimental food as the respective control groups (red, green and blue solid circles), as indicated by the fact that they are on long food rails labelled "open-ended", but nonetheless are calorically restricted (do not move as far along their respective rails as controls) because of the dietary dilution.

Experiments combining qualitative restriction by balance and dilution provide a powerful approach for exploring the detailed effects of diets on life history and other responses. This is demonstrated in the comparison of the red, green and blue treatment pairs in Fig. 3A. The two solid black negatively-sloped diagonal lines, which are energy isolines (i.e., all points falling on each of these lines represent equal energy intakes), show that the three unrestricted groups have the same caloric intakes and the restricted groups eat 60% of this. In terms of caloric restriction the red, green and blue treatment pairs are thus equivalent. At the level of nutrient intakes, however, the treatment pairs are fundamentally different. In the green pair, the 40% reduction of calories is split equally between protein and carbohydrate (green dashed lines labelled P− and C−). In contrast, in the blue comparison protein is restricted to a substantially greater degree than is carbohydrate (blue P− is longer than blue C−), whereas in the red comparison carbohydrate is restricted

to a greater degree than is protein (red C − vs. red P −). Using a nutrient space to systematically define treatment differences in this way can thus help to disentangle the effects of caloric intake and specific nutrients on responses of interest, such as longevity.



3.2. Restriction relative to what?

Systematic emulation of nutritional scenarios (variation in food quality and quantity) and its impacts on diet, as described above, is an important step for defining the ecological causes of variation in animal life history responses. To integrate evolution into the model, however, it needs to be expanded beyond ecological variation to explicitly represent also the interests of the animal. This is done by conceptualising the manipulations not primarily as a comparison between intakes in the restricted and control treatment groups (i.e., *treatment-based* comparisons, as in Fig. 3A), but between each of these and the intake target (i.e., a *target-based* comparisons). As illustrated in Fig. 3B, target-based comparisons provide a very different perspective on how we might interpret the effects of nutritional variation on lifespan and other fitness-related responses.

As in Fig. 3A, in all three comparisons shown in Fig. 3B the 60% group is calorie restricted, whereas the control group is not (intakes of the controls fall on the same energy isoline as the intake target). In the green comparison, the intake point for the control group coincides with the intake target - i.e., they achieve their target intake for both macronutrients - and the calorie-restricted treatment experiences an approximately equal reduction of protein and carbohydrate intake. In the blue comparison, however, *both* groups are restricted in carbohydrate intake, with the 60% group being more highly restricted than controls (the blue vertical dashed and solid lines, respectively). For protein, the blue 60% group is not restricted at all (it ingests the target level of protein), whereas the control group ingests an *excess* of protein (blue P +). Conversely, in the red comparison both groups are restricted in protein intake (red P −), albeit the control group to a lesser extent, but the 60% group meets its carbohydrate target while controls ingest a carbohydrate excess (red C +).

The implications of such target-based analyses for reconciling results of dietary restriction experiments with evolutionary theory are fundamental. An essential starting point for this reconciliation is to distinguish cases where the restricted animals actually are restricted in the intake of a specific nutrient relative to their evolved priorities (i.e., the intake target) whereas the controls are not (e.g., green in Fig. 3B). This is very different from cases where both groups are restricted to differing extents (e.g., carbohydrate for the blue treatment), or where the "restricted" group is not restricted in relation to the nutrient but rather rescued from an excess that is ingested by the controls (e.g., protein for the blue treatment). Furthermore, since these relationships are sensitive to the position of the

Fig. 3. Geometric model distinguishing different dietary restriction scenarios and their consequences for nutrient intake. Dash-lined and solid-lined circles represent restricted and control (unrestricted) treatment groups, respectively. The solid black lines (diagonal in A. and B., horizontal in C.) are energy isolines, such that all points that lie on any one of these represent the same energy intake. Calorie intake therefore changes along the protein:carbohydrate nutritional rails ("calorie vector" in A.), and macronutrient ratio changes across the rails ("balance vector"). A. In treatment-based contrasts, a comparison is made between nutrient intakes of the control and restricted groups. Three protocols of dietary restriction are shown. In quantitative restriction (white symbols), restricted animals are provided with reduced food rations (the short dashed dark nutritional rail) relative to ad libitum-fed controls (the long solid dark rail, representing provision of food in excess). In qualitative restriction by dilution (colored symbols), both the restricted and control groups are fed ad libitum (represented as long rails), but the diet of the controls is diluted to restrict the distance moved along the nutritional rail. Since the control and diluted food within each treatment pair (color) have the same macronutrient ratio, the rails for the two foods are overlaid and thus appear as one. In qualitative restriction by balance, comparisons are made between groups fed foods that differ in nutrient balance (blue vs. green vs. red). Colored dashed lines labelled P- and C- show the reduction in protein intake and carbohydrate intake, respectively, experienced by the calorie restricted group relative to the same-colored control group. B. In target-based contrasts, comparisons are made not primarily between restricted and control groups, but between each of these and the intake target (IT). Dashed and solid colored lines show macronutrient intakes of restricted and control groups, respectively, relative to the intake target (P- and C- = protein and carbohydrate deficit, and P+ and C+ = protein and carbohydrate excess). C. Logic of qualitative restriction by dilution, using the green treatment contrast to illustrate. The target region for macronutrient intakes is narrow compared with the diluent (in this case water). Whereas the control treatment achieves its target intake both for macronutrients and water, the restricted treatment achieves the target for water but is restricted in calorie (and therefore macronutrient) intake.

target, this approach can be used to factor variance in nutrient requirements (e.g., due to thermodynamic demands) into models of the relationship between diet and lifespan. This might be a particularly relevant consideration for interpreting the results of laboratory experiments on the relationship between diet and aging (Gibbs and Smith, 2016).

3.3. Quantitative restriction or qualitative restriction by dilution?

Manipulations along the balance vector (Fig. 3A) can only be achieved by varying diet composition (qualitative variation), as discussed above. A noteworthy question, however, is whether and in which circumstances manipulations along the calorie vector should be achieved by experimentally varying the amount of food available (quantitative restriction, white contrast in Fig. 3A) or the concentration of nutrients (qualitative restriction by dilution), or some combination of these. The red, green and blue contrasts in Fig. 3A and B assumed the latter approach, as this standardised in an important respect between the restricted and unrestricted groups, namely all had ad libitum access to foods. This is a relevant concern, because variation in the temporal availability of foods (hence the pattern of feeding) has itself been associated with changes in lifespan (Mattson et al., 2014) and health (Solon-Biet et al., 2015b; Zarrinpar et al., 2016), independent of caloric intake.

On the other hand, from an experimental perspective neither is qualitative restriction by dilution free of the potential for confound, because animals in the diluted treatments often partially compensate for the reduced nutrient concentration of the foods by increasing consumption, and in so doing ingest greater quantities of the diluent than do the controls (Simpson et al., 2015). This can, however, be dealt with. One way is to dilute nutrients using a filler that is neutral over the relevant range with respect to the response of interest. This is shown schematically in Fig. 3C in relation to the green treatment from Fig. 3B. In this hypothetical example water has been used as the diluent in the restricted group, because the effect of increased water intake on fitness (including lifespan) is negligible compared with reduced macronutrient intake. Geometrically, this is expressed as an asymmetry in the shape of the intake target, such that the target encompasses a wide range of water intakes compared with macronutrient intakes. Accordingly, intakes of both groups fall within the target range for water, but only the unrestricted group falls within the target range for macronutrients. A second way to deal with effects of a diluent follows straightforwardly from this; namely, include it in the model as an axis in its own right. In the spirit of nutritional geometry, this has the advantage that, rather than merely controlling for any direct effects of the filler, it can provide additional information about the actual mechanisms through which diets influence lifespan.

Important as these issues are, we should be equally vigilant to pay attention to the *ecological* significance of experimental design. From this perspective, all three manipulations are valid, because each relates to a different ecological scenario. Quantitative restriction emulates a shortage of food overall, qualitative restriction by balance emulates a shortage of nutritionally balanced foods, and qualitative restriction by dilution emulates a situation where animals have access only to foods with low nutrient concentrations. The appropriate experiment will depend on what is considered to be the combination of scenarios to which the species has been exposed within the relevant ecological context.

We suspect, however, that in the majority of cases qualitative dietary variation (both in terms of nutrient balance and concentration) will have played an important role, either on its own or in conjunction with quantitative variation. This is because when faced with shortages of preferred foods, most animals shift to lower quality alternatives, which are nutritionally imbalanced, have low overall nutrient concentrations, defensive chemicals or some combination of these. This has been studied in the wild most intensively in primatology, where such foods are referred to as “fallback foods” (Lambert and Rothman, 2015), but has also been recorded for a wide range of other taxa and contexts (e.g., Foster, 1977; Fredriksson et al., 2006; Tait et al., 2014; Remonti et al., 2015). Falling back to lower quality foods is also predicted by mechanistic theory (Simpson and Raubenheimer, 1996), and is likely the reason that animals have evolved physiological and associated behavioral mechanisms to ameliorate the impacts of eating nutritionally imbalanced and dilute foods (i.e., rules of compromise, and intake compensation respectively) rather than reject these outright (Raubenheimer and Simpson, 1997). Substantial lab-based evidence comes from nutritional geometry experiments in which animals are confined to a single nutritionally-imbalanced and/or diluted diet; the fact that all animals studied to date, spanning diverse taxa from insects to humans, including model species used in aging research (*Drosophila* and laboratory mice, see below), eat those foods rather than reject them (Simpson and Raubenheimer, 2012), strongly suggests that they would do likewise in the wild. The extent to which quantitative restriction acts alone in the wild, or is combined with qualitative restriction (i.e., overall food shortage), is an issue that needs to be dealt with on a case-by-case basis. When they do co-occur, the relevant experiment will combine quantitative and qualitative restriction, as done by Solon-Biet et al. (2015b) using laboratory mice.

4. From manipulations to responses

Regardless of which variety of dietary restriction is emulated in experiments, a crucial step in nutritional geometry is to quantify the effects of these manipulations on the functional and mechanistic responses of interest. This is done by expressing the response levels (e.g., lifespan or circulating IGF1) using response surface methodology (Simpson et al., 2004; Lee et al., 2008).

As illustrated in Fig. 4, using data for a predatory beetle (Jensen et al., 2012), this approach provides a model in which the nutritional environment is emulated in a multi-dimensional context to generate a spread of nutritional states (Fig. 4A) to which outcomes of interest can be related and compared with theoretical models (Fig. 4B). In this case, the model is constructed around protein and lipid, which together comprise total macronutrients for the animals in this experiment, because carbohydrates play at most a very minor role in the diets of obligate predators (Eisert, 2011). Intake points were spread laterally by confining different experimental groups to one of a range of foods varying in protein:lipid ratios (qualitative variation by balance), each of which is represented in the figure as a different food rail (radial line). Quantitative restriction was used to achieve variation in the amounts of each food (hence calories) eaten, where one group was provided ad libitum access and other groups 66%, 50% or 33% of the amount eaten by the ad libitum group. This experiment therefore emulated a situation where variability in food quality and availability interact to influence functional outcomes, as is considered to be the case for predators in the wild (Tait et al., 2014; Kohl et al., 2015).

In Fig. 4B we have superimposed on the experimentally-generated intakes a response surface for egg production by the beetles, as well two measures of the animals' homeostatic regulation, the selected intake target (unconstrained priority) and the rule of compromise (regulation in the face of nutritional imbalance). The rule of comprise was generated using only the intakes of the experimental groups that were provided food ad libitum, because the intake points of the quantitative-restricted groups would, for obvious reasons, not provide a measure of homeostatic regulation.

Several points of interest emerge from this figure. It shows, firstly, that there was a distinct peak for maximal egg production, demonstrating that both macronutrient balance and amount are important for reproduction. Secondly, when given the option, these beetles combined nutritionally complementary foods in the correct proportions to compose a diet that maximized egg production. Third, animals confined to nutritionally imbalanced foods which prevented them from reaching the intake target (and hence attaining maximal egg production) regulated their intake in a pattern that was not consistent with calorie prioritisation (dashed line a), protein prioritisation (dashed line b), or fat prioritisation (dashed line c). Rather, they showed an intermediate pattern in which both nutrients were under- and over-ingested to some extent, with fat intake being held more constant than protein. A

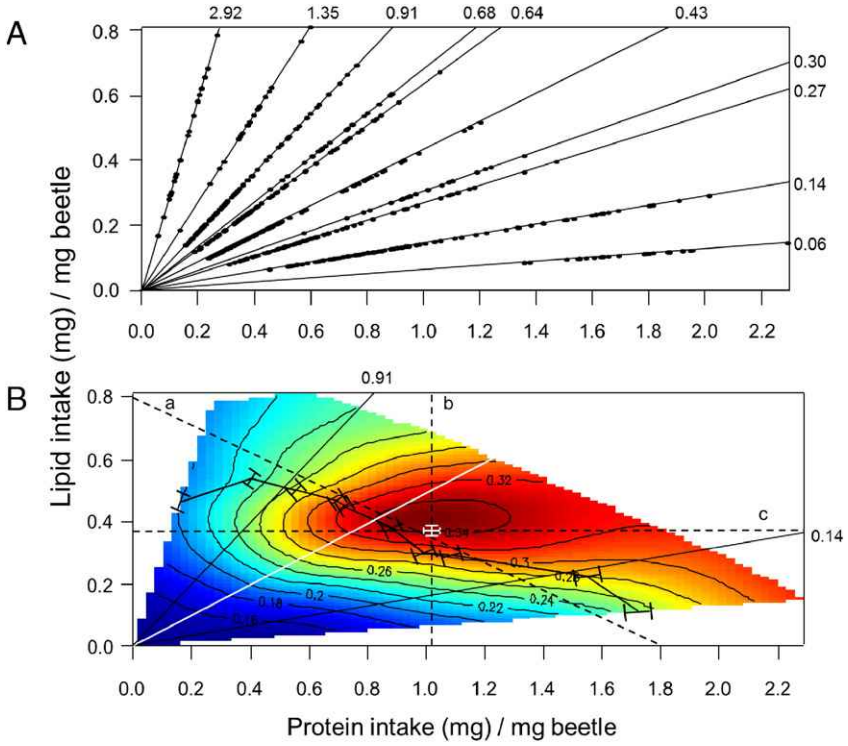


Fig. 4. Geometric model describing the relationship between the nutritional environment, homeostatic regulation, and fertility in the predatory beetle *Anchomenus dorsalis*. **A.** Each point represents the intake across the experiment of a single beetle. Intakes were spread across the nutrient space using foods spanning a range of fat:protein rails (2.92–0.06), and spread along each rail by varying the levels of dietary restriction (ad libitum feeding, or 66%, 50 or 33% of ad libitum). **B.** Response surface showing the relationship between the nutrient intakes shown in A. and egg production. Egg production increases from blue to red. The white cross is the bivariate mean intake point (±S.E.) selected by the beetles when given access to two nutritionally complementary foods (F:C ratio 0.91 and 0.14, plotted as solid food rails). This was significantly different to the expected outcome if feeding on the two foods was random (the solid white line). The radially projecting truncated bars represent the mean ± S.E. intakes of the experimental groups that were allowed ad libitum access to one of the range of experimental foods differing in the F:C ratio. The error bars coincide with the respective nutritional rails, because the intakes were constrained to vary along the nutritional rails. The rule of compromise is shown by the solid line joining these no-choice intakes. Dashed lines show various hypothetical outcomes, as in Fig. 2B: a. fixed energy intake; b. fixed protein intake; and c. fixed fat intake. Modified from Jensen et al. (2012).

functional explanation for this pattern is suggested by the relationship between the mean intake points and the contours on the response surface. For each diet, with the exception of the extreme high-protein diet, the animals fed to the point on their respective food rails that was closest to the highest-valued contour that was accessible on their respective food rail; in other words, where they could not maximize egg production through selecting an intake target, the beetles regulated to the point of intake that provided the highest fecundity that was possible given the imbalanced diet to which they were confined.

This example is provided as an illustration of how the Geometric Framework can be used to explore the links between ecological variation (quantitative and qualitative food constraint), homeostatic responses (intake targets and rules of compromise) and a functional outcome (egg production). We have deliberately chosen an example involving reproductive output rather than lifespan, because reproduction provides a more direct proxy for evolutionary fitness than does lifespan, thus allowing us to show how the animal's regulatory responses (selection of intake targets and rules of compromise) mediate between food quality and fitness. Several studies, however, have used this approach to examine the relationships between diet, longevity and reproduction.

5. Geometry, diet, longevity and reproduction

The effects of dietary macronutrients on aging, lifespan and reproduction have been investigated using the Geometric Framework in several species of insects and one mammalian model, C57BL6 mice. These studies have recently been reviewed (Solon-Biet et al., 2015c; Le Couteur et al., 2016), and rather than repeat that here we will provide a brief summary using select examples.

Overall, studies combining qualitative restriction by balance with qualitative restriction by dilution, as illustrated in (Fig. 3), have shown that low-protein high-carbohydrate diets fed ad libitum are associated with increased lifespan; total caloric intake, in itself, had either no effect or was correlated negatively with lifespan (Lee et al., 2008; Skorupa et al., 2008; Jensen et al., 2015; Fanson et al., 2009; Fanson and Taylor, 2012; Harrison et al., 2014; Solon-Biet et al., 2014). Additionally, several of these studies showed that diets that improved reproductive performance were higher in protein and lower in carbohydrate than diets associated with longer lifespans, and when given the choice of intake, animals tended to select diets that favoured reproduction over lifespan. Fig. 5, for example, shows for field crickets (*Teleogryllus commodus*; Maklakov et al., 2008) and *Drosophila melanogaster* (Jensen et al., 2015) that both male and female animals have different nutritional optima for lifespan and sex-specific measures of reproductive fitness, which suggest differential selection pressures acting upon the sexes (Archer and Hunt, 2015). Males and females of both species survived longer on high-carbohydrate low-protein diets. Interestingly, diets that maximized male lifespan were similar to what maximized lifetime calling effort (crickets) and lifetime reproductive output (flies); however, diets that maximized female lifetime egg production were higher in protein and lower in carbohydrate than those that maximized lifespan. When offered a choice between complementary foods, sexes of both species selected feeding trajectories towards optimizing reproduction, but neither sex reached their nutritional optima for lifetime reproductive output. One interpretation of this is that dietary regulation is

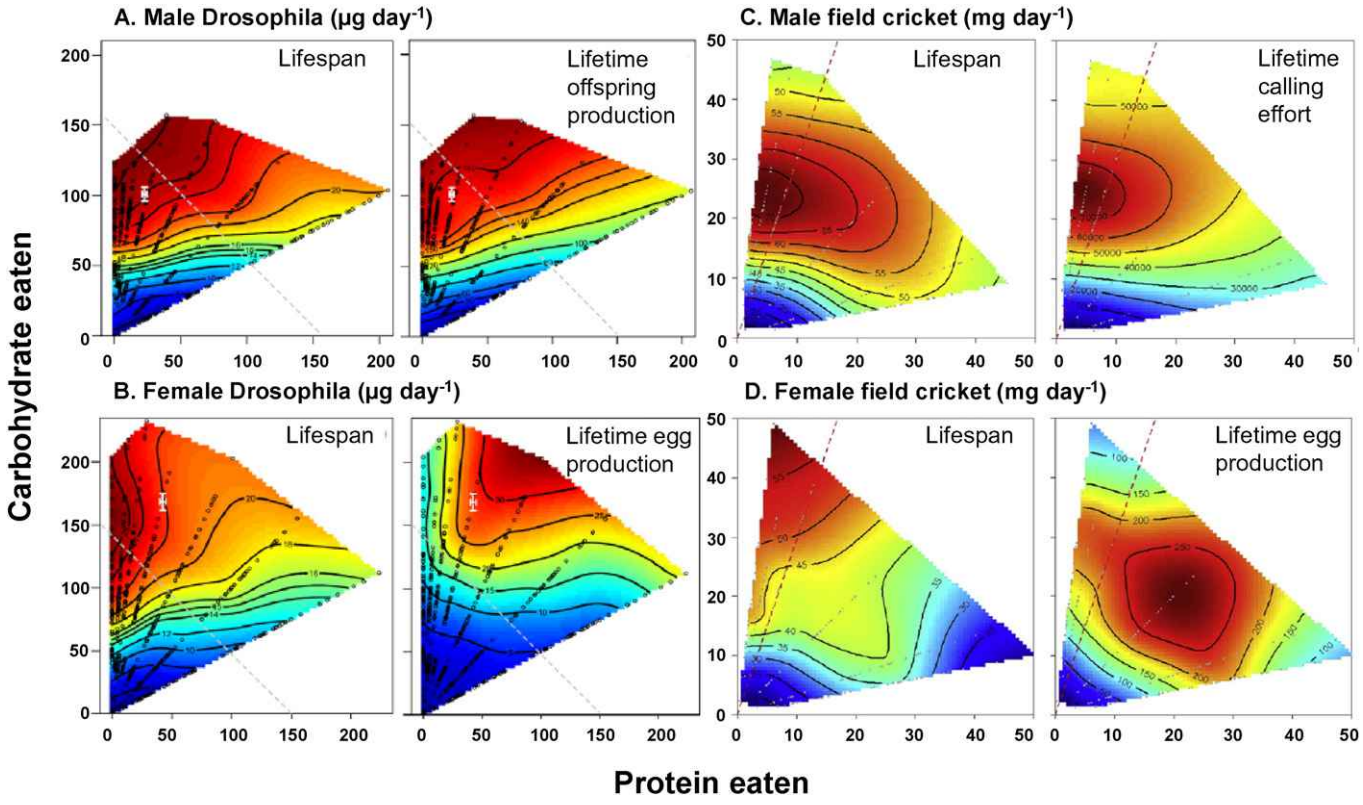


Fig. 5. Non-parametric thin-plate spline response surfaces depicting the effects of carbohydrate and protein intake on lifespan and reproduction in two insect species, *Drosophila melanogaster* and field crickets (*Teleogryllus commodus*). Insects were given ad libitum access to one of 29 (*Drosophila*) or 24 (field cricket) diets varying in the ratio of protein to carbohydrate. Plots of *Drosophila* include the regulated intake point (±SE) for flies offered a choice of complementary foods, which is shown in white. Response surfaces rise in strength from blue to red. Figures are from Jensen et al. (2015; *Drosophila*) and Maklakov et al. (2008; field crickets).

constrained by intralocus sexual conflict over nutritional optimization (Maklakov et al., 2008; Adler and Bonduriansky, 2015).

Similar to studies of insects, geometric experiments have demonstrated that dietary macronutrient balance has profound effects on lifespan and reproductive performance in mice, with these traits being optimized on different diet compositions (Solon-Biet et al., 2014, 2015a). Longevity in mice was maximized on low-protein high-carbohydrate diets (P:C ratio of 1:13 for males and 1:11 for females). Reproductive performance, in contrast, was best on diets higher in protein for males, and likewise for some responses for females. In males, testes mass and sperm counts were higher on diets with a P:C ratio of 1:1, as were uterine mass and ovarian follicle number (3:1) in female mice; however, other female reproductive measures were optimized at lower ratios of P:C, including estrous cycling (1:8), and corpora lutea count (1:11).

In addition to lifespan and reproduction, Solon-Biet et al. (2014, 2015a) measured physiological responses of the mice to dietary macronutrient manipulation, including circulating amino acids and hepatic mechanistic target of rapamycin (mTOR) activation, and used response surfaces to relate these to macronutrient intake (Fig. 6). The analysis showed that circulating branched-chain amino acids, but not other amino acids, were increased at greater dietary protein:carbohydrate ratios, and corresponded well with the patterns of mTOR activation and life history responses. This example provides an illustration of how geometric analysis can be used to generate models that provide insight into links between the nutritional environment, mechanism, and function.

6. Homeostasis, hypotheses and aging research

To this point we have emphasised the importance in nutritional ecology of systematically generating variance in nutrition, and measuring the consequences of such variation, for example in terms of reproduction and lifespan. We have also demonstrated that animals are not passive transducers of environmental circumstances to functional outcomes, but show intricate regulatory responses that mediate these relationships to their advantage (Fig. 4). Our aim in this section is to consider more explicitly the role that these responses can play in helping to generate testable hypotheses to advance aging research.

6.1. Intake targets

One way that animals actively mediate between their nutritional environments and performance is through choosing foods and regulating amounts eaten to compose a diet that maximises fitness — i.e., selecting an intake target. Relating the position of this target to response surfaces representing functional outcomes can provide insight into the links between functional and mechanistic responses of the animal.

For example, the demonstration by Lee et al. (2008) that *Drosophila* fruit flies select an intake target that coincides with lifetime egg production, but not longevity, suggests that the former has played a greater role in the evolutionary shaping of the regulatory systems (as would be expected under Darwinian theory). It might, on the other hand, be demonstrated in some cases that the selected intake point does *not* coincide with a peak in lifetime reproduction (Simpson et al., 2004). This would lead us to suspect that at least one important factor has been omitted from the model. It could, for example, be that the animal has evolved under high risk of predation, and selects a diet that minimises development time at a cost to fertility, as was suggested to be the case by Rodrigues et al. (2015) for *Drosophila* larvae. Alternatively, it might select a diet that reduces fertility but supports longevity beyond its reproductive

lifespan — i.e., menopause, as in humans and some cetaceans (Brent et al., 2015). This would suggest the hypothesis that kin selection has played a role in the evolution of lifespan, via resource transfer to offspring or grandchildren (Lee, 2003). Such discrepancies between expectation and observation thus provide powerful guides to direct research for understanding the evolution of aging.

6.2. Rules of compromise

A second measure of homeostasis that the Geometric Framework brings into aging research is the rule of compromise. As discussed above, this represents a regulatory trade-off between the costs of over-ingesting some nutrients and under-ingesting others in circumstances where ecological constraint prevents the animal from reaching its intake target. In common with intake targets, such responses have likely evolved under natural selection to achieve specific functional outcomes, albeit under nutritional constraint. Rules of compromise, too, can therefore provide links between the evolution of life histories, ecology and the behavioral and physiological mechanisms that the animal uses to positively influence outcomes in the face of an uncertain environment. We already have given an example illustrating this, where the rule of compromise measured for the predatory beetles studied by Jensen et al. (2012) corresponded with the highest fertility that was achievable on each experimental food rail (Fig. 4).

To explore these points further, in Fig. 7 we have redrawn the response surface for lifetime egg production by *Drosophila* females from the study of Lee et al. (2008). The grey dots represent intakes by individual flies, generated through the use of diets with different macronutrient ratios (variation across the food rails) and different degrees of dietary dilution using water (variation along each food rail). Although the rule of compromise was not reported in that experiment, it can readily be ascertained from the plot. Considering that the flies that moved furthest along their respective nutritional rails (i.e., those on more concentrated foods) had the option to limit intake to any lower level than they actually achieved, their realised intakes can be considered a regulated response, in contrast with flies on the more dilute treatments which were constrained by dietary dilution to achieve lower macronutrient intakes. The only other explanation is that they were over-ingesting macronutrients to compensate for the reduced water content of the nutrient-concentrated diets; this is, however, highly unlikely because nutrient-free water was available separately throughout the experiment. The rule of compromise is thus represented by the curve joining the groups of high intake points across all of the rails, each group being approximated in the figure by an (arbitrarily-sized) ellipse.

As in the beetle experiment, this illustrates that the flies that were least constrained in the distance they could move along their respective rails achieved the highest egg production of all flies on that rail, suggesting a functional reason the rule of compromise is shaped as it is. It is important to note that other than these fitness considerations (i.e., the shape of the response surface), there is no obvious reason to expect that the rule should take this form. Conversely, given the shape of the response surface, it would be surprising to discover that the rule of compromise took any other form (Simpson et al., 2004). But if it did, as discussed in relation to the intake target above, the mismatch between expectation and observation can provide powerful leads for understanding the links between ecology, nutrition and life history.

For example, a rule of compromise called protein prioritisation (PP), in which protein intake is maintained constant in the face of dietary macronutrient imbalance while non-protein energy (carbohydrates and fats) varies (as in the vertical red-brown array Fig. 2B), has been observed in spider monkeys (Felton et al., 2009b) and humans (Gosby et al., 2013). In Fig. 7 we have superimposed a vertical line on the response surface for egg production, representing the PP model. The expected intakes if the flies followed this rule are given by the filled circles (here we have plotted only expected intakes on the three diets with protein:carbohydrate ratios higher than the target ratio, because expected intakes on the low-protein diets fall beyond the y-axis scale). The primary difference between PP and the observed compromise rule is that protein is over-consumed relative to the intake target (the grey-shaded ellipse) in the observed pattern, as shown by the yellow arrow, but not the PP pattern. Functionally, the model shows that any mutant flies that followed the PP rule would achieve considerably lower fertility than the wild-type flies, and would likely be strongly selected against.

In some circumstances, however, PP could plausibly evolve in flies, as it has in humans and spider monkeys. For example, if in a particular ecological context the increased time costs or risk of predation involved in moving further along the nutritional rails outweighed the fertility benefits, then individuals with genetic mutations for PP may have increased fitness and the strategy would spread within the population. Similarly, immune responses (Ponton et al., 2011) might be better optimized under the PP rule, and in some environments the benefits of pathogen resistance could more than counter-balance the fertility costs of reduced protein intake. Alternatively, mutations at the level of nutrient metabolism might explain the shift in the cost-benefit matrix that is associated with the evolution of a changed rule of compromise. In humans, for example, ingested protein excesses can be toxic, with intakes above approximately 35% of total energy intake potentially being lethal (Bilsborough and Mann, 2006). High protein intakes, particularly when paired with low carbohydrate intakes, also influence nutrient-sensing pathways in ways that are associated with metabolic disorders and accelerated aging (Fig. 6; Solon-Biet et al., 2014).

In overview, measures of homeostatic responses can provide a powerful tool for understanding the links between nutrition and functional responses such as lifespan and reproduction. They provide a direct indication of which combination of energy, specific nutrients or nutrient balance the animal itself prioritises, taking the guesswork out of identifying the relevant nutritional dimensions to target in a given species. In so doing, they narrow the search space for discovering crucial information that is needed to integrate across different areas of aging research.

7. Discussion

The number of theories that has been put forward to explain aging has been estimated to exceed 300 (Bilinski et al., 2015). Although not all of these are extant, and with some addressing mechanism and others evolution, neither are they all competing theories. Nonetheless, this bears testimony to a highly active research field that is probing questions of considerable theoretical and practical importance. It might also reflect a field that is missing some key information and ideas needed to reconcile or decide among the theories, and which suffers from poorly defined terminology (Hayflick, 2007a, b).

The dominant experimental model is caloric restriction, in which energy per se (the undifferentiated mix of macronutrients) is considered the nutritional component that links diet with lifespan (Masoro, 2005; Speakman and Mitchell, 2011; Ravussin et al., 2015). There is substantial empirical support for the link between energy intake and lifespan in a range of organisms, and yet there is no unified theory to explain this. One reason, as discussed in the Introduction, is that evolutionary and mechanistic aging research have not been well integrated. In this paper we have suggested that another, more fundamental reason which might in itself explain the lack of unification, is that aging research has drawn only minimally on the body of theory that links animal nutrition to evolution via ecology, nutritional ecology.

We have presented an approach from nutritional ecology, nutritional geometry, which we suggest can help to consolidate aging research. The distinctive feature is that it enables the different energetic components of the diets – the macronutrients – to be distinguished, and their individual and interactive effects on animals' responses quantified. Both theory (Raubenheimer and Simpson, 2016) and abundant empirical evidence (Simpson and Raubenheimer, 2012) suggest that the behavior, physiology and life history responses of animals are

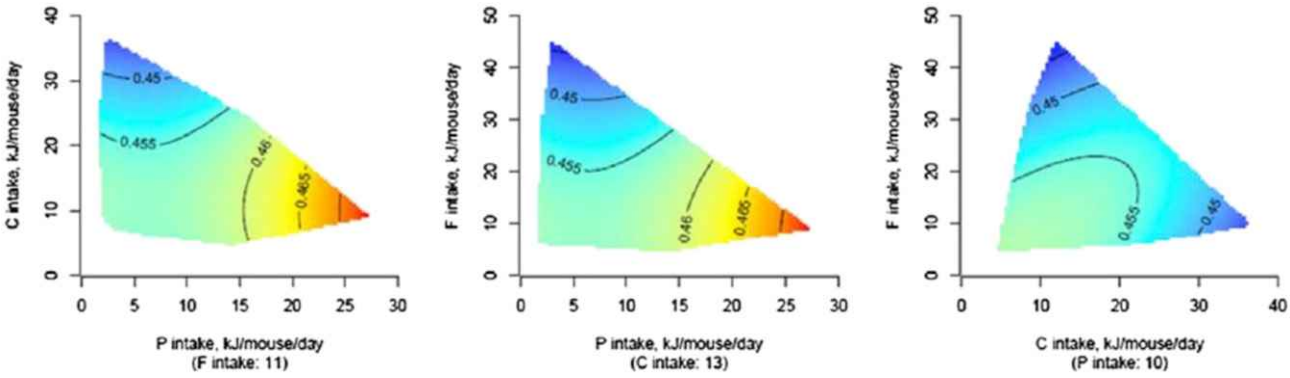


Fig. 6. Non-parametric thin-plate spline response surface for effects of macronutrient intake on mTOR activation for mice after 15 months of ad libitum feeding on one of 25 diets (from Solon-Biet et al., 2014). In this experiment fat, carbohydrate and protein were varied independently. Pairwise relationships between the three nutrients and mTOR activation are shown, in each case with the third nutrient fixed at its median value. mTOR activation was measured as the ratio of phosphorylated (pmTOR) to total mTOR. Response surfaces rise in strength from blue to red.

sensitive to specific blends of energetic macronutrients. Examples we have presented here include diet selection (intake targets), responses to nutritional constraint (rules of compromise), physiological responses (mTOR) and life history responses (fecundity and lifespan). We have also shown how expressing these different facets of the animal-environment interaction in this way enables them to be interrelated within a single model showing, for example, how diet selection relates to fecundity (Fig. 4) and how nutrition mediates the relationship between fecundity and lifespan (Fig. 5). This, in turn, provides a framework to generate and test specific mechanistic, ecological and evolutionary hypotheses about how the different components of the system fit together (Fig. 7).

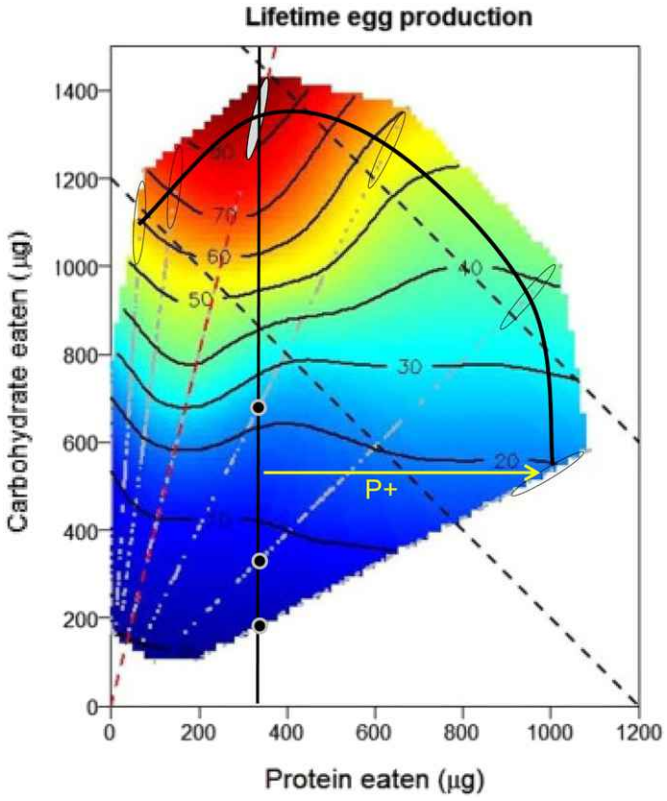


Fig. 7. Geometric model linking fertility to homeostatic regulation. The response surface represents lifetime egg production by female *Drosophila melanogaster*. The black arc represents the rule of compromise, estimated using the intakes of the flies that were able to feed to the furthest point along the respective nutritional rails (each group highlighted using an arbitrarily-sized oval). The grey-filled oval is on the target nutritional rail, as demonstrated in the same experiment using a protocol similar to that shown in Fig. 1B. The solid dots show expected intakes if the flies followed the protein prioritisation rule (vertical black line, as detailed in Fig. 2B). The yellow arrow labelled P+ shows the extent to which protein was over-ingested by flies on the most protein-rich experimental diet. See text for further details. Data are from Lee et al. (2008).

The multiple-nutrient approach does not, of course, necessarily contradict the widely observed relationship between calorie intake and lifespan; rather, our point is that it can help to explain that relationship. One possibility, as is commonly assumed, is that the caloric restriction effect on lifespan is causally driven by total calorie intake, rather than specific nutrients or the impact of quantitative restriction on the temporal pattern of feeding (discussed above). From a nutritional ecology perspective, however, this is a surprising idea, because any dietary currency around which credible evolutionary theories of aging are formed would need to be substantively linked to fitness, and yet, as we discuss further below, it is in most cases difficult to link calorie intake to fitness in any way that is more substantial than correlation. If the association is, indeed, correlative, then the problem that aging research faces is that energy as a nutritional currency will provide at best weak links between diet and both evolution and the mechanisms of aging, making it near impossible to reconcile mechanistic and evolutionary theories of how diet relates to aging. For example, as mentioned above, the effects of quantitative dietary restriction on lifespan (Mattson et al., 2014) and health (Solon-Biet et al., 2015b; Zarrinpar et al., 2016) can be achieved through altering the temporal pattern of food availability, even when the restricted group shows no reduction in calorie intake relative to the ad libitum group. If the altered feeding pattern that almost invariably accompanies quantitative dietary restriction is the causal link with lifespan, rather than restricted calorie intake per se (Simpson et al., 2015), then a search for mechanisms or evolutionary explanations involving calorie intake will be diversionary. On the other hand, a dietary currency that is both associated with lifespan and substantively linked to fitness would provide a powerful common guide for evolutionary and mechanistic theories of aging, and hence a template for integrating the two.

Examples such as those presented in this paper show that calories per se is not a concept that resonates well with the ways that animals respond to nutrition (see also Simpson and Raubenheimer, 2012). We also know from animal physiology that all calories are not equal: animals need protein, fats and carbohydrates in specific proportions to fulfil different needs, one of which is the provision of calories to fuel energy metabolism. Functionally, therefore, different caloric substrates have different significance for the animal, and combining these into an undifferentiated pool of energy is unlikely to provide a metric of diet that relates in a reliable way to fitness. For example, if an animal has a dietary requirement of 1000 cal per day, it clearly matters what proportion of this is available as amino acids to fund structural growth and enzyme synthesis, and

what is available to fuel energy metabolism. Furthermore, as mentioned above, specific macronutrients eaten in excess can have negative impacts on fitness, even if total caloric intake does not exceed the optimal requirement (e.g., the red and blue solid circles in Fig. 3B both meet target energy requirements, and yet are associated with ingested surpluses of carbohydrate and protein, respectively). Therefore, calories from different macronutrients, even if ingested in the same meal, can relate to fitness with opposite sign — in some contexts more is better and in others more is worse. It thus seems unlikely that fitness-relevant responses, including feeding behavior, lifespan and reproduction, would be linked in any substantive way to calorie intake per se. Figs. 4 and 7, on the other hand, clearly show how nutrient balance is a property of diet that predicts fitness, and that the animals' behavior is sensitive to this link, whereas in these examples calorie intake relates directly neither to behavior nor fitness.

It might, alternatively, be argued that it is not energy per se that is the crucial nutritional link to fitness, but specifically that proportion of dietary macronutrient that is allocated to generating ATP to fuel energy metabolism. This is a valid hypothesis, and in circumstances where animals are limited primarily by substrate for energy metabolism, it is perhaps even a likely one. However, we are here entering the realm of nutrient balance. Different animals require different combinations of macronutrients to fuel energy metabolism - for example obligate predators such as cats are minimally able to utilise carbohydrate (reviewed in Kohl et al., 2015), and there is an upper limit of approximately 35% of calories from protein beyond which humans are unable to extract energy, regardless of the total intake (Bilsborough and Mann, 2006). Therefore, if metabolically available energy were the causal link between diet and fitness-related responses such as reproduction and lifespan, then information about dietary macronutrient balance, and not just total calorie intake, would be needed to predict these responses.

Nutritional ecology theory therefore does not anticipate that in general calories per se can provide a direct guide for understanding the lifespan extension observed in aging research based on quantitative dietary restriction, but rather that in most cases calorie intake is a proxy for other factors that link diet with lifespan. A possible exception is for animals that are adapted to a diet that varies little with respect to macronutrient balance - i.e., a restricted spread across the balance vector (Fig. 3A). In such cases caloric intake could provide a sufficiently reliable proxy for the intake of the required macronutrient blend that macronutrient-specific nutrient sensing mechanisms would be redundant and energy intake could provide a reliable measure of nutritional state (Simpson and Raubenheimer, 2001). This would predict that the life-extending effects of caloric restriction (either by quantitative restriction or qualitative restriction by dilution) should be more pronounced in dietary specialists than generalists. It might also explain why model animals for aging research appear to be more sensitive to caloric restriction than non-model species (Nakagawa et al., 2012), because a long history of successive generations feeding on invariant laboratory diets can result in laboratory animals developing characteristics associated with extreme nutritional specialism (Warbrick-Smith et al., 2009).

If it is correct that the commonly observed link between calorie intake and lifespan is due to correlation rather than causation, then nutritional ecology can make a substantial contribution in helping to unravel the links between diet and biological aging, through identifying the individual and interactive roles of specific nutrients and disentangling these from other causes (e.g., the temporal pattern of feeding). If it turns out to be wrong, nutritional ecology would benefit from broadening its tenets to accommodate the caloric perspective. Either way, a priority for aging research is to move beyond theory and experimental protocols that are restricted to a given dietary component, whether that be energy or protein. Rather, an open-ended approach is needed, to establish which dimension(s) of diet, if any (e.g., Adler and Bonduriansky, 2014), actually do drive life extension in dietary restriction, and whether and how these differ with circumstances and between species. It is possible, for example, that a diversity of responses will be uncovered as the range of non-model species examined expands (e.g., Nakagawa et al., 2012), but the significance of this will be obscured if we focus too narrowly on specific nutritional currencies. Integrating within the multi-nutrient context behavioral, physiological, ecological and life history responses, as illustrated in this paper, could help the field move closer to a unified theory of biological aging.

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References

Adler, M.I., Bonduriansky, R., 2014. Why do the well-fed appear to die young? BioEssays 36, 439–450.
Adler, M.I., Bonduriansky, R., 2015. Sexual conflict, life span, and ageing. Cold Spring Harb. Perspect. Biol. http://dx.doi.org/10.1101/cshperspect.a017566.
Archer, C.R., Hunt, J., 2015. Understanding the link between sexual selection, sexual conflict and aging using crickets as a model. Exp. Gerontol. 71, 4–13.
Bilinski, T., Paszkiewicz, T., Zadrag-Tecza, R., 2015. Energy excess is the main cause of accelerated aging of mammals. Oncotarget 6, 12909–12919.
Bilsborough, S., Mann, N., 2006. A review of issues of dietary protein intake in humans. Int. J. Sport Nutr. Excerc. Metab. 16, 129–152.
Brent, L.J.N., Franks, D.W., Foster, E.A., Balcomb, K.C., Cant, M.A., Croft, D.P., 2015. Ecological knowledge, leadership, and the evolution of menopause in killer whales. Curr. Biol. 25, 746–750.
Calow, P., 1987. Towards a definition of functional ecology. Funct. Ecol. 1, 57–61.
Cesari, M., Vellas, B., Gambassi, G., 2013. The stress of aging. Exp. Gerontol. 48, 451–456.
Chambers, P.G., Simpson, S.J., Raubenheimer, D., 1995. Behavioural mechanisms of nutrient balancing in *Locusta migratoria*. Anim. Behav. 50, 1513–1523.
Cheplick, G.P., 2015. Plant Evolutionary Ecology. Oxford University Press, Oxford, New York.
Dev, S.B., 2015. Unsolved problems in biology—the state of current thinking. Prog. Biophys. Mol. Biol. 117, 232–239.
Eisert, R., 2011. Hypercarnivory and the brain: protein requirements of cats reconsidered. J. Comp. Physiol. B. 181, 1–17.
Fanson, B.G., Taylor, P.W., 2012. Protein:carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. Age (Dordr.) 34, 1361–1368.
Fanson, B.G., Weldon, C.W., Perez-Staples, D., Simpson, S.J., Taylor, P.W., 2009. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). Aging Cell 8, 514–523.
Felton, A.M., Felton, A., Wood, J.T., Foley, W.J., Raubenheimer, D., Wallis, I.R., Lindenmayer, D.B., 2009a. Nutritional ecology of *Ateles chamek* in lowland Bolivia: how macronutrient balancing influences food choices. Int. J. Primatol. 30, 675–696.
Felton, A.M., Felton, A., Raubenheimer, D., Simpson, S.J., Foley, W.J., Wood, J.T., Wallis, I.R., Lindenmayer, D.B., 2009b. Protein content of diets dictates the daily energy intake of a free-ranging primate. Behav. Ecol. 20, 685–690.
Flatt, T., Heyland, A., 2011. Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs. Oxford University Press.
Flatt, T., Schmidt, P.S., 2009. Integrating evolutionary and molecular genetics of aging. Biochim. Biophys. Acta Gen. Subj. 1790, 951–962.
Fletcher, Q.E., Selman, C., 2015. Aging in the wild: insights from free-living and non-model organisms. Exp. Gerontol. 71, 1–3.
Fontana, L., Partridge, L., 2015. Promoting health and longevity through diet: from model organisms to humans. Cell 161, 106–118.
Foster, M.S., 1977. Ecological and nutritional effects of food scarcity on a tropical frugivorous bird and its fruit source. Ecology 58, 73–85.
Evolutionary Ecology. In: Fox, C.W., Roff, D.A., Fairbairn, D.J. (Eds.), Concepts and Case Studies. Oxford University Press, Oxford, New York.
Fredriksson, G.M., Wich, S.A., Trisno, 2006. Frugivory in sun bears (*Helarctos malayanus*) is linked to el Nino related fluctuations in fruiting phenology, East Kalimantan, Indonesia. Biol. J. Linn. Soc. 89, 489–508.
Gibbs, V.K., Smith, D.L., 2016. Nutrition and energetics in rodent longevity research. Experimental Gerontology. http://dx.doi.org/10.1016/j.exger.2016.04.004.
Gosby, A.K., Conigrave, A.D., Raubenheimer, D., Simpson, S.J., 2013. Protein leverage and energy intake. Obes. Rev. 15, 183–191.
Harrison, S.J., Raubenheimer, D., Simpson, S.J., Godin, J.G., Bertram, S.M., 2014. Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. Proc. Biol. Sci. 281, 1792.
Hayflick, L., 2007a. Biological Aging Is no longer an Unsolved Problem. In: Rattan, S.I.S., Akman, S. (Eds.), Biogerontology: Mechanisms and Interventions, pp. 1–13.
Hayflick, L., 2007b. Entropy explains aging, genetic determinism explains longevity, and undefined terminology explains misunderstanding both. PLoS Genet. 3, 2351–2354.

Hughes, K.A., Reynolds, R.M., 2005. Evolutionary and mechanistic theories of aging. Annu. Rev. Entomol. 50, 421–445.
Irwin, M.T., Raharison, J.-L., Raubenheimer, D., Chapman, C.A., Rothman, J.M., 2015. The nutritional geometry of resource scarcity: effects of lean seasons and habitat disturbance on nutrient intakes and balancing in wild sifakas. PLoS One 10, e0128046.
Jensen, K., Mayntz, D., Toft, S., Clissold, F.J., Hunt, J., Raubenheimer, D., Simpson, S.J., 2012. Optimal foraging for specific nutrients in predatory beetles. Proc. R. Soc. B 279, 2212–2218.
Jensen, K., McClure, C., Priest, N.K., Hunt, J., 2015. Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in *Drosophila melanogaster*. Aging Cell 14, 605–615.
Johnson, C.A., Raubenheimer, D., Rothman, J.M., 2013. 30 days in the life: daily nutrient balancing in a wild chacma baboon. PLoS One 8, e70383.
Kirkwood, T.B.L., 1977. Evolution of aging. Nature 270, 301–304.
Kohl, K.D., Coogan, S.C.P., Raubenheimer, D., 2015. Do wild carnivores forage for prey or nutrients? Evidence for nutrient-specific foraging in vertebrate predators. BioEssays 37, 701–709.
Krebs, C.J., 2015. One hundred years of population ecology: successes, failures and the road ahead. Integr. Zool. 10, 233–240.
Lambert, J.E., Rothman, J.M., 2015. Fallback foods, optimal diets, and nutritional targets: primate responses to varying food availability and quality. Annu. Rev. Anthropol. 44, 493-512.
Le Couteur, D.G., Solon-Biet, S., Cogger, V.C., Mitchell, S.J., Senior, A., de Cabo, R., Raubenheimer, D., Simpson, S.J., 2016. The impacts of low protein, high carbohydrate diets on ageing and lifespan. Cell. Mol. Life Sci. 73, 1237–1252.
Lee, R.D., 2003. Rethinking the evolutionary theory of aging: transfers, not births, shape social species. Proc. Natl. Acad. Sci. U. S. A. 100, 9637–9642.
Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. Proc. Natl. Acad. Sci. U. S. A. 105, 2498–2503.
Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessman, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., Brooks, R.C., 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr. Biol. 18, 1062–1066.
Masoro, E.J., 2005. Overview of caloric restriction and ageing. Mech. Ageing Dev. 126, 913–922.
Mattson, M.P., Allison, D.B., Fontana, L., Harvie, M., Longo, V.D., Malaisse, W.J., Mosley, M., Notterpek, L., Ravussin, E., Scheer, F.A., Seyfried, T.N., Varady, K.A., Panda, S., 2014. Meal frequency and timing in health and disease. Proc. Natl. Acad. Sci. U. S. A. 111, 16647–16653.
McCay, C., Crowell, M., Maynard, L., 1935. The effect of retarded growth upon the length of life and upon ultimate size. J. Nutr. 10, 63–79.
McLachlan, A.J., Ladle, R.J., 2011. Barriers to adaptive reasoning in community ecology. Biol. Rev. 86, 543–548.
Monaghan, P., Charmantier, A., Nussey, D.H., Ricklefs, R.E., 2008. The evolutionary ecology of senescence. Funct. Ecol. 22, 371–378.
Nakagawa, S., Lagisz, M., Hector, K.L., Spencer, H.G., 2012. Comparative and meta-analytic insights into life-extension via dietary restriction. Aging Cell 11, 401–409.
Nussey, D.H., Froy, H., Lemaitre, J.-F., Gaillard, J.-M., Austad, S.N., 2013. Senescence in natural populations of animals: widespread evidence and its implications for bio-gerontology. Ageing Res. Rev. 12, 214–225.
Partridge, L., Gems, D., 2006. Beyond the evolutionary theory of ageing, from functional genomics to evo-gero. Trends Ecol. Evol. 21, 334–340.
Piper, M.D.W., Partridge, L., Raubenheimer, D., Simpson, S.J., 2011. Dietary restriction and aging: a unifying perspective. Cell Metab. 14, 154–160.
Ponton, F., Wilson, K., Cotter, S.C., Raubenheimer, D., Simpson, S.J., 2011. Nutritional immunology: a multi-dimensional approach. PLoS Pathog. 7, e1002223.
Raubenheimer, D., Boggs, C.L., 2009. Nutritional ecology, functional ecology and functional ecology. Funct. Ecol. 23, 1–3.
Raubenheimer, D., Simpson, S.J., 1993. The geometry of compensatory feeding in the locust. Animal Behaviour 45, 953–964.
Raubenheimer, D., Simpson, S.J., Mayntz, D., 2009. Nutrition, ecology and nutritional ecology: toward an integrated framework. Functional Ecology 23, 4–16.
Raubenheimer, D., 2011. Toward a quantitative nutritional ecology: The right-angled mixture triangle. Ecological Monographs 81, 407–427.
Raubenheimer, D., Simpson, S.J., 1997. Integrative models of nutrient balancing: application to insects and vertebrates. Nutr. Res. Rev. 10, 151–179.
Raubenheimer, D., Simpson, S.J., 2016. Nutritional ecology and human health. Annu. Rev. Nutr. 36. http://dx.doi.org/10.1146/annurev-nutr-071715-051118 (in press).
Raubenheimer, D., Lee, K.P., Simpson, S.J., 2005. Does Bertrand's rule apply to macronutrients? Proc. R. Soc. B 272, 2429–2434.
Raubenheimer, D., Machovsky-Capuska, G.E., Chapman, C.A., Rothman, J.M., 2015. Geometry of nutrition in field studies: an illustration using wild primates. Oecologia 177, 223–234.
Raubenheimer, D., Simpson, S.J., Mayntz, D., 2009. Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct. Ecol. 23, 4–16.
Ravussin, E., Redman, L.M., Rochon, J., Das, S.K., Fontana, L., Kraus, W.E., Romashkan, S., Williamson, D.A., Meydani, S.N., Villareal, D.T., Smith, S.R., Stein, R.I., Scott, T.M., Stewart, T.M., Saltzman, E., Klein, S., Bhapkar, M., Martin, C.K., Gilhooly, C.H., Holloszy, J.O., Hadley, E.C., Roberts, S.B., for the CALERIE Study Group, 2015. A 2-year randomized controlled trial of human caloric restriction: feasibility and effects on predictors of health span and longevity. J. Gerontol. A Biol. Sci. Med. Sci. 70, 1097–1104.
Remonti, L., Balestrieri, A., Raubenheimer, D., Saino, N., 2015. Functional implications of omnivory for dietary nutrient balance. Oikos 000, 001–008. http://dx.doi.org/10.1111/oik.02801.
Rodrigues, M.A., Martins, N.E., Balancé, L.F., Broom, L.N., Dias, A.J., Fernandes, A.S., Rodrigues, F., Sucena, É., Mirth, C.K., 2015. *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. J. Insect Physiol. 81, 69–80.
Rothman, J.M., Raubenheimer, D., Chapman, C., 2011. Nutritional geometry: gorillas prioritize non-protein energy while consuming surplus protein. Biol. Lett. 7, 847–849.
Shokhirev, M.N., Johnson, A.A., 2014. Effects of extrinsic mortality on the evolution of aging: a stochastic modeling approach. PLoS One 9, e86602.
Simpson, S.J., Raubenheimer, D., 1996. Feeding behaviour, sensory physiology and nutrient feedback: a unifying model. Entomol. Exp. Appl. 80, 55–64.
Simpson, S.J., Raubenheimer, D., 2005. Obesity: the protein leverage hypothesis. Obesity Reviews 6, 133–142.
Simpson, S.J., Raubenheimer, D., 2001. A framework for the study of macronutrient intake in fish. Aquac. Res. 32, 1–12.
Simpson, S.J., Raubenheimer, D., 2007. Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 62, 707–713.
Simpson, S.J., Raubenheimer, D., 2009. Macronutrient balance and lifespan. Aging 1, 875–880.
Simpson, S.J., Raubenheimer, D., 2012. The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity. Princeton University Press.
Simpson, S.J., Le Couteur, D.G., Raubenheimer, D., 2015. Putting the balance back in diet. Cell 161, 18–23.
Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., Raubenheimer, D., 2004. Optimal foraging when regulating intake of multiple nutrients. Anim. Behav. 68, 1299–1311.
Skorupa, D.A., Dervisefendic, A., Zwiener, J., Pletcher, S.D., 2008. Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. Aging Cell 7, 478–490.
Solon-Biet, S.M., Mitchell, S.J., Coogan, S.C.P., Cogger, V.C., Gokam, R., McMahon, A.C., Raubenheimer, D., de Cabo, R., Simpson, S.J., Le Couteur, D.G., 2015b. Dietary protein to carbohydrate ratio and caloric restriction: comparing metabolic outcomes in mice. Cell Rep. 11, 1–6.
Solon-Biet, S.M., Mitchell, S.J., de Cabo, R., Raubenheimer, D., Le Couteur, D.G., Simpson, S.J., 2015c. Macronutrients and caloric intake in health and longevity. J. Endocrinol. 226, R17–R28.
Solon-Biet, S.M., Walters, K.A., Simanainen, U.K., McMahon, A.C., Ruohonen, K., Ballard, J.W.O., Raubenheimer, D., Handelsman, D.J., Le Couteur, D.G., Simpson, S.J., 2015a. Macronutrient balance, reproductive function, and lifespan in ageing mice. Proc. Natl. Acad. Sci. U. S. A. 112, 3481–3486.
Solon-Biet, S.M., McMahon, A.C., Ballard, W.O., Ruohonen, K., Wu, L.E., Cogger, V.C., Warren, A., Huang, X., Pichaud, N., Melvin, R.G., Gokarn, R., Khalil, M., Turner, N., Cooney, G.J., Sinclair, D.A., Raubenheimer, D., Le Couteur, D.G., Simpson, S.J., 2014. The ratio of macronutrients, not caloric intake, dictates cardio metabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab. 19, 418–430.
Speakman, J.R., Mitchell, S.E., 2011. Caloric restriction. Mol. Asp. Med. 32, 159–221.
Stroud, J.T., Bush, M.R., Ladd, M.C., Nowicki, R.J., Shantz, A.A., Sweatman, J., 2015. Is a community still a community? Reviewing definitions of key terms in community ecology. Ecol. Evol. 5, 4757–4765.
Tait, A.H., Raubenheimer, D., Stockin, K.A., Merriman, M., Machovsky-Capuska, G.E., 2014. Nutritional geometry and macronutrient variation in the diets of gannets: the challenges in marine studies. Mar. Biol. 161, 2791–2801.
Warbrick-Smith, J., Raubenheimer, D., Simpson, S.J., Behmer, S.T., 2009. Three hundred and fifty generations of extreme food specialization: testing predictions of nutritional ecology. Entomol. Exp. Appl. 132, 65–75.
Zarrinpar, A., Chaix, A., Panda, S., 2016. Daily eating patterns and their impact on health and disease. Trends Endocrinol. Metab. 27, 69–83.